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SOILS AND BIOGEOCHEMISTRY
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DAVIS, CALIFORNIA 95616-8627

May 14,2000

CALFED Bay-Delta Program
1416 Ninth St., Suite 1155
Sacramento. CA 95814

Dear Colleague,

It is our pleasure to present for your consideration the proposal titled " Evaluation of Biological Assimilatory Capacity for Mechanism-Based Adaptive Management for Selenium in the San Franciso Bay-Delta", Dr. Teresa W-M. Fan, lead PI.

With regards to the "Threshold Requirements", the PSP Cover Sheet is found at the beginning of the proposal, while the Environmental Compliance Checklist, Land **Use** Checklist, and the State and Federal contract forms are attached at the back as per instructions. Please note that there are no letters of notification. since this is a research project that does "not include any physical action on the mound", as stated in the CALFED 2001 PSP, p. 50.

Please also note that in Section H, Compliance with Standard Terms and Conditions, there is a letter addressing the Univ. of California, Davis position on the terms.

Sincerely,

Teresa W-M. Fan, FT

Attachment H

Proposal # 2001- F209 (Office Use Only)

PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: **Evaluation** of Biological Assimilatory Capacity **for** Mechanism-Based Adaptive Management for Selenium in the San Francisco Bay-Delta

Applicant Name: University of California-Davis

Contact Name: Dr. Teresa W-M. Fan

Mailing Address: Dept of Land, Air and Water Resources, One Shields Ave., Univ. of California, Davis, CA 95616

Telephone: 530/752-1450

Fax: 530/752-1552

Email: twfan@,ucdavis.edu

Amount of funding requested $ 651,288

Some entities charge different costs dependent on the source of the funds. If it is different for state or federal funds list below.

State cost 651,288, based on 10% indirect costs Federal cost 871,559, based on 46.5-48.5% indirect costs

Cost share partners? ____ Yes X No

Identify partners and amount contributed by each ____

Indicate the Topic for which you are applying (cheek **only** one box).

- ☐ Natural Flow Regimes
- ☐ Nonnative Invasive Species
- ☐ Channel Dynamics/Sediment Transport
- ☐ Flood Management
- ☐ Shallow Water Tidal/ Marsh Habitat
- ☒ Contaminants
- ☐ Beyond the Riparian Corridor
- ☐ Local Watershed Stewardship
- ☐ Environmental Education
- ☐ Special Status Species Surveys and Studies
- ☐ Fishery Monitoring, Assessment and Research
- ☐ Fish Screens

What county or counties is the project located in? Yolo

What CALFED ecozone is the project located in? See attached list and indicate number. Be as specific as possible Project is located in Ecozone 10, while results are applicable to Ecozones 1, 2, and 11-14

Indicate the type of applicant (check only one box):

- ☐ State agency
- ☐ Public/Non-profit joint venture
- ☐ Local government/district
- ☒ University
- ☐ Other: ____
- ☐ Federal agency
- ☐ Non-profit
- ☐ Tribes
- ☐ Private party

Indicate the primary species which the proposal addresses (check **all that apply):**

- ☐ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☐ Winter-run chinook salmon
- ☐ Spring-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Fall-run chinook salmon
- ☐ Delta smelt
- ☐ Longfin smelt
- ☒ Splittail
- ☐ Steelhead trout
- ☐ Green sturgeon
- ☐ Stripedbass
- ☐ White Sturgeon
- ☐ All chinook species
- ☐ Waterfowl and Shorebirds
- ☐ All anadromous salmonids
- ☐ Migratory birds
- ☐ American shad
- ☑ Other listed T/E species: Proposed study is mechanistic and apulicable to all fish species

Indicate the type of project (check only one box):

- ☑ Research/Monitoring
- ☐ Watershed Planning
- ☐ Pilot/Demo Project
- ☐ Education
- ☐ Full-scale Implementation

Is this a next-phase of an ongoing project? Yes ____ No X
Have you received funding from CALFED before? Yes ____ No X

If yes, list project title and CALFED number_____

Have you received funding from CVPIA before? Yes ____ No X

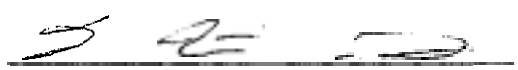
If yes, list CVPIA program providing funding, project title and CVPIA number (if applicable):

_____

By signing below, the applicant declares the following:

- The truthfulness of all representations in their proposal;
- The individual signing the form is entitled to submit the application on behalf of the applicant [if the applicant is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

Teresa W-M. Fan
Printed name of applicant


Signature of applicant

B. EXECUTIVE SUMMARY

Evaluation of Biological Assimilatory Capacity for Mechanism-Based Adaptive Management *for* Selenium in the San Franciso Bay-Delta

Amount Requested: $651,288 based on 10% indirect costs for California Resource Agency funds

Teresa W-M. Fan (lead-PI), Dept of Land, Air and Water Resources, Univ. of California, One Shields Ave., Davis, CA 95616 ph 530/752-1450, fax 530/752-1552, twfan@ucdavis.edu
with Richard M. Higashi, Crocker Nuclear Laboratory, and Swee J. Teh, Dept of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, Univ. of California, Davis

Selenium (Se) contamination is probably one of the best known cases that has led to serious population decline of aquatic top predators such as waterfowl and fish in a number of watersheds, and currently threatens key fish species in the Bay-Delta, such as the splittail (CALFED, 2000). The historical lessons of Se pollution around the world underscore the urgent need for early-warning indicators of environmental deterioration in a given watershed. Unfortunately, no such indicators are known. Chemical analysis alone cannot uncover such indicators, due to the extensive transformations, foodchain bioavailability, biogeochemistry, and unknown toxicity mechanism(s) of Se.

For these reasons, total waterborne Se – while readily analyzed – is widely considered to be an unreliable indicator of a toxic risk to upper trophic organisms such as fish. This fact has been documented in numerous scientific publications (please see Project Description). Furthermore, this fact is behind the recent EPA Great Lakes ruling (EPA, 1996), and constitutes a primary conclusion of the EPA Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation (EPA Office of Water, 1998). This fact is also reflected in the California Toxics Rule (EPA, 1997) suggesting site-specific Se criteria. CALFED summarized this state of knowledge, stating that *"A question has been raised over the adequacy of concentration-based standards.... EPA has convened a nine-member panel in a Peer-Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation that is investigating the need for differentiating the toxicity of different forms of selenium and developing site-specific objectives for selenium."* (CALFED-306, 1999). Thus, there are abundant indications that the regulations may align closer to the a t o x i c facts in the near future.

The complex biogeochemistry, biological transformations, and foodchain accumulation of the currently unknown ecotoxic form(s) of Se are all components that determine the biological assimilatory capacity (BAC) in Se-laden aquatic systems. Therefore, the most useful Se risk indicator would be one that *can* gauge exceedence of BAC.

Consequently, this proposal will address the following objectives:

(1) Probe the a t o x i c mechanisms underlying Se impact on indigenous aquatic wildlife of contaminated watersheds connecting to the Bay-Delta, using state-of-the-science biogeochemical and cellular biomarker tools;

(2) Utilize results from Objective 1 to uncover biochemical forms of Se with the potential to be assayed conveniently, which *can* then be deployed as an early warning tool for impending Se ecotoxicity;

(3) Test these indicators in field studies, with aim of assessing exceedance of biological assimilatory capacity (BAC) on a site-specific basis.

In order to achieve these objectives, we propose a work plan that, in essence, will test the hypothesis that protein-bound Se forms in intermediate food-chain organisms are an indicator of BAC exceedance. This will consist of biochemically and histologically probing the mechanisms of toxicity in two species of indigenous fish, bluegills that are known to be sensitive to Se impact and a Federally listed threatened species, Sacramento splittail, determining the biochemical forms of Se that are transferred from food to fish to cause toxicity, and confirming these relationships in field studies involving the same fish species. If successful, the proposed approach should help bridge major gaps in our understanding of Se biogeochemistry and ecotoxicology while facilitating the choice of management options and implementation of a more flexible and reliable policy for Se discharge limits.

C. PROJECT DESCRIPTION

C.1. STATEMENT OFTHEPROBLEM

C.1.a. Problem

Se contamination is probably one of the best known cases that has led to serious population decline of aquatic top predators such as waterfowl and fish in a number of watersheds. The historical lessons of Se pollution underscore the urgent **need** for early-waming indicators of environmental deterioration in a given watershed. Unfortunately, no such indicators are known. Chemical analysis alone cannot uncover such indicators, due to the extensive transformations, foodchain bioavailability, biogeochemistry, and unknown toxicity mechanism(s) of Se.

For these reasons, total waterborne Se – while readily analyzed, and thus widely used – is equally widely considered to be an unreliable indicator of ecotoxic risk to upper trophic organisms such as fish. This fact has been documented in numerous scientific publications (please see Conceptual Model section below), and clearly spelled out in CALFED's own document (CALFED-306, 1999), an excerpt of which is reproduced in the Executive Summary. A leading scientist in this area recently stated that ***"...measures of waterborne selenium alone would be inadequate for assesssing toxic risk."*** (Skorupa, 1998). Furthermore, this fact is behind the EPA Great Lakes ruling (EPA, 1996), and constitutes a primary conclusion of the EPA Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation (EPA, 1998). This fact is also reflected in the California Toxics Rule (EPA, 1997) suggesting site-specific Se criteria. Thus, there are abundant indications that the regulations may align closer to the atoxic facts in the near future. Therefore, effective management of waterways for ecological effects of Se contamination cannot continue to depend on total Se chemical analysis alone.

C.1.b. Conceptual Model

Despite the last two decades of research effort, it is still unknown as to what are the early indicators of Se impact that ***can*** be reliably applied to different ecosystems. Although waterborne Se concentration and total Se body burden of top predators and foodchain organisms have been utilized for Se risk assessment, none of these parameters were consistently reliable and applicable on a site-specific (e.g. lentic versus lotic) basis (Lemly, 1993; Canton and Van Derveer, 1997; Adams et al., 1997; Hamilton et al., 1997). This is primarily a result of the complex biogeochemistry of Se and extensive foodchain transformations (US EPA Office of Water, 1998), which have eluded a fundamental understanding of both the ecotoxic (foodchain-mediated) and toxic (organismal) mechanism(s) that underlie the teratogenic effects and reproductive failure observed for Se-inflicted top predators.

The complexity of the compartments which drive the biogeochemical transformations are illustrated in Figure 1 (adapted from Cooke and Bruland, 1987). In both natural and Se-contaminated waters, the dominant forms of dissolved Se are reportedly selenite (+4 oxidation state) and/or selenate (+6 oxidation state) (e.g. Cooke and Bruland, 1987). There **are** also dissolved organoselenium form(s) present in the water column, but the chemical nature of these forms is largely unknown and their concentrations are generally much lower than those of the inorganic Se forms.

In spite of the low concentrations, the organoselenium form(s) may still play a very important role in Se atoxic effects (e.g. Rosetta and Knight, 1995; Besser et al., 1993). The dissolved selenium oxyanions are primarily taken up by aquatic producers including algae and bacteria (process ***a / a'),*** and biotransformed into organoselenium form(s) and elemental selenium (Se^0) (process *i*). Once accumulated in the aquatic producers, Se ***can*** be transferred through various aquatic consumers (e.g. zooplankton, insect larvae, larval fish, bivalves, etc.) into the top predators such as waterfowl and piscivorous fish (process ***k).*** Se biomagnification and further transformation ***can*** occur during this foodchain transfer process (Maier and Knight, 1994). However, the actual Se biotransformation products and the specific form(s) transferred up the foodchain that cause toxicity in aquatic ecosystems are poorly understood.

The aquatic producers and other planktonic organisms are also the basis for detrital materials which *can* settle onto the sediment (process]) and become the **food** source for sediment organisms (process ***k).*** In addition to this Se input into the sediment, waterbnne selenite and selenate ***can*** be physically adsorbed onto the sediment particles, ingested, absorbed, and transformed by the sediment organisms (process 1/ *l'*). **As** such, **sediments act as a sink for waterborne Se.** Sediment-bund selenate and selenite *can* be reduced to insoluble Se^0 by anaerobic microbial activities (process *i'*). This and water column-derived Se^0 *can* be reduced further to selenide ***(-2*** form) (process *n)* and/or reoxidized to selenite and selenate (process *m* / *m'*) by microorganisms in the sediment and/or in the **guts** of sediment macroinvertebrates. Selenides *can* enter the foodchain via absorption into sediment organisms (process *o*) or be oxidized to selenite and selenate (process *p*). Selenium of different oxidation states *can* be further biotransformed by sediment organisms and transferred up the foodchain (process ***k).*** Selenium biotransformation, bioaccumulation, and transfer through both sediment and water column foodwebs constitute the major path for ecotoxic risk in aquatic ecosystems.

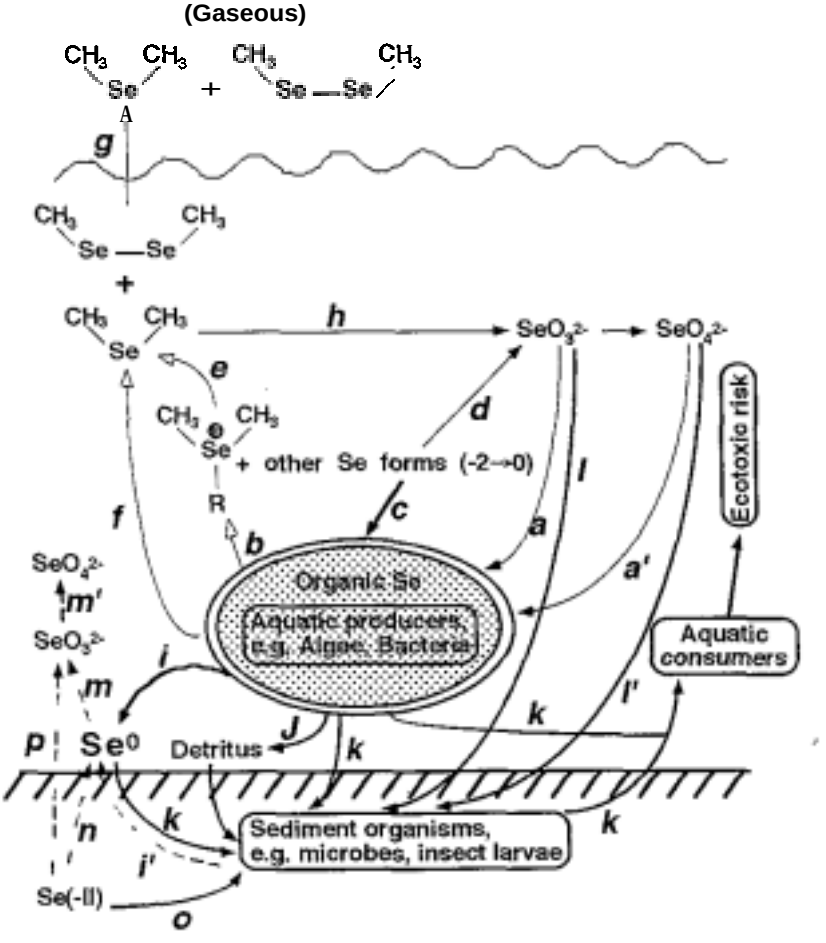


Figure 1. Current understanding of Se biogeochemical cycling. This scheme is modified from Cooke and Bruland (1987) with the main addition of the foodchain transfer pathways (in solid arrows). The dissipation pathways involving Se volatilization are indicated with open arrows

In addition to accumulating Se into the biomass, the aquatic producers may be the main drivers for the volatilization of Se via the production of methylated selenides including dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) (process *f*). These methylated selenides ***can*** be oxidized to selenite (process *h)* or exit the water column into the atmosphere (process **g).** Se volatilization into the atmosphere may represent an important process via which a significant loss of Se occurs in some aquatic systems (Fan et al., 1998b). Methylated selenides *can* also be generated from dissolved selenonium precursor(s) (process ***e)*** released by aquatic producers into the water (process ***b).*** Moreover, other organoselenium forms ***can*** be released into the water by aquatic producers and are reoxidized (process *d*) to selenite and/or reabsorbed by aquatic organisms (process *c*).

From Figure 1 and the above discussion, it is clear that *biological assimilation* constitutes a major part of the Se biogeochemical cycling and *is the key to Se impact on biota.* Therefore, the biological

assimilatory capacity (BAC) for Se is an indication as to how much Se contamination a given watershed *can* tolerate. Unfortunately, due to the complexity of the processes involved, BAC for Se cannot be readily assessed from simple water and sediment parameters, nor from existing chemical analysis of Se speciation. The complexity is also the origin of the sitedependence of biological impacts. This is a consensus opinion from a recent EPA Peer-Consultation workshop (US EPA Office of Water, 1998). On the other hand, it is reasonable to assume that Se BAC is modulated by both dissipation (volatilization and Se^0 precipitation) and foodchain transfer pathways. The dissipation pathways lead to Se loss from the water while the foodchain transfer pathways indicate ecotoxic risk. Since Se^0 *can* be re-assimilated (albeit more slowly) directly or indirectly by the biota (see Figure 1), the only route of net loss to the system is typically Se volatilization. Thus, Se BAC for a given system should be largely determined by the two processes of Se volatilization and foodchain transfer capacity; this proposal deals with the latter.

Of course, from a practical standpoint, it would be extremely difficult to measure the true BAC on a site-specific basis. However, it may be feasible to develop convenient and reliable indicator for an early warning of BAC overload. Such development would require a fundamental understanding of the Se ecotoxic mechanism(s), e.g. the Se form(s) that are biotransformation products of aquatic producers, transferred up the foodchain, and linked to Se toxicity in top predators. There are hints that proteinaceous forms of Se, in particular selenomethionine (Se-Met) in the protein, may be an important ecotoxic form.

Se-Met, supplemented as a free amino-acid form in diets of laboratory feeding studies, has been shown to cause similar toxic symptoms in avian species as those observed in the field (e.g. Heinz et al., 1988 & 1989). Hence, free Se-Met is often considered to have similar "potency" as the true (but currently unknown) ecotoxic form. As a micronutrient, Se is primarily metabolized into selenoamino acids, and subsequently incorporated into proteins (Stadtman, 1996; Ganther, 1974). Studies conducted in our laboratory and elsewhere have shown that proteinaceous Se-Met is the major transformation product of microalgae (Fan et al., 1998a & b; Wrench, 1978; Bottino et al., 1984). Proteinaceous Se-Met was also the major form in field-collected deformed embryos and macroinvertebrates (Fan, Higashi, and Skorupa, unpublished results). These findings strongly suggest the need for a systematic investigation of the role of proteinaceous Se-Met in Se foodchain transfer and toxicity.

C.1.c. Hypothesis Being Tested

Summarizing the above, chemical measurements alone - such as the current practice of total waterborne Se - have severe limitations as indicators of biological or ecosystem impacts. We propose that exceedance of BAC would be one useful indicator of ecosystem impact (currently, there are no such indicators), which should be assessable through a combination of biochemical and histological analyses. Non-funded efforts are currently underway in our research group to test the expert-panel consensus hypothesis (EPA, 1998 and references cited therein) that the proteinaceous Se in food items (e.g. water column and benthic invertebrates) may be a good measure of upper-trophic-level ecotoxic risk. This is due to the high Se concentmtions typically found in protein, coupled with its high nutritional availability to the next trophic level. The first study of this type has been submitted by us for publication (Fan et al., submitted to Aquatic Toxicology).

This proposal will address the following objectives:

(1) Probe the ecotoxic mechanisms underlying Se impact on indigenous aquatic wildlife of contaminated watersheds connecting to the Bay-Delta, using state-of-the-science biogeochemical and cellular biomarker tools;

(2) Utilize results from Objective 1 to uncover biochemical forms of Se with the potential to be assayed conveniently, which *can* then be deployed as an early warning tool for impending Se ecotoxicity;

(3) Test these indicators in field studies, with aim of assessing exceedance of biological assimilatory capacity (BAC) on a site-specific basis.

The three objectives will be fulfilled through two tasks: Task 1 is centered around analysis of biochemical forms of Se, and Task 2 is fish histopathology to assess the biological impacts.

C. 1.d. Adaptive Management

Despite the last two decades of research effort, it is still unknown as to what are the early indicators of Se impact that can be reliably applied to different ecosystems. Although waterborne Se concentration and total Se body burden of top predators and foodchain organisms have been utilized for Se risk assessment, none of these parameters were consistently reliable and applicable on a site-specific (e.g. lentic versus lotic) basis (Lemly, 1993; Canton and Van Derveer, 1997; Adams et al., 1997; Hamilton et al., 1997). This is primarily a result of the complex biogeochemistry of Se and extensive foodchain transformations (US EPA Office of Water, 1998), which have eluded a fundamental understanding of both the ecotoxic (foodchain-mediated) and toxic (organismal) mechanism(s) that underlie the teratogenic effects and reproductive failure observed for Se-inflicted top predators. Since ecotoxic or toxic mechanism(s) are likely to be similar in top predators, the mechanistic understanding acquired by this project should be generally applicable at most sites where contamination and related environmental compliance and regulatory issues are of a concern to the CALFED.

We envision that the methods developed here, which is to gauge exceedance of BAC, will be used in field surveys and monitoring, for the express purpose of updating decisions based on adaptive management. According to a CALFED document (CALFED *306,* 1999), "A *question has been raised over the adequacy of concentration-based standards.... EPA has convened a nine-member panel in a Peer-Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation that is investigating the need for differentiating the toxicity of different forms of selenium and developing site-specific objectives for selenium."* The principal investigator of this proposal, Dr. Teresa Fan, is one of the EPA panelists that is defining what will be the adaptive management shift from the current Se concentration standards to more relevant biochemical indicators. Thus, the proposed research aims to bridge major gaps in our understanding of Se biogeochemistry and ecotoxicology, which are needed to choose between management options, eventually leading to implementation of a more flexible policy for Se discharge limits.

C.1.e. Educational Objectives

This section is not applicable, since this proposal does not have "primarily education focus".

C.2. PROPOSED SCOPE OF WORK

C.2.a. Geographic Boundaries of Project

This project will be conducted at the University of California at Davis, in Yolo County. The results of this work are applicable to Ecozones 1, 2, and 11-14.

C.2.b. Approach

To achieve the objectives (stated above in section C.1.c.), we propose a work plan that, in essence, will test the hypothesis that protein-bound or other Se forms in intermediate foodchain organisms are an indicator of BAC exceedance. This will consist of probing the mechanisms of toxicity in two species of indigenous fish (the endangered split tail and bluegill that is known to be sensitive to Se impact), determining the biochemical forms of Se that are transferred from food to fish to cause toxicity, and confirming these relationships in field studies involving the same fish species.

Objective 1: Probe the ecotoxic mechanisms underlying Se impact on indigenous aquatic wildlife of contaminated watersheds connecting to the Bay-Delta, using state-of-the-science biogeochemical and cellular biomarker tools.

Among Se-laden watersheds, California's San Joaquin River watershed has been highly impacted, and consists of some of the best-documented cases of Se ecotoxicity. In this system, as with others, it is clear that reproductive impairment and teratogenesis are typically the most sensitive endpoints observed for Se toxicosis in both waterfowl (Ohlendorf et al 1986; Hoffman et al 1998; cf. Frankenberger and

Engberg, 1998) and fish species (Saiki and Ogle, 1995; Coyle et al 1993; Lemly, 1993, 1997). However, it is unclear whether the same ecotoxic mechanism(s) govern these effects in both categories of top predators. Relatively, much less is known about the mechanism(s) for aquatic fish, which is a reason for the proposed focus. Presently, we are pursuing similar investigations on impacted waterfowl species in collaboration with Drs. J. Skorupa (U.S. Fish and Wildlife Service) and M. Fry (UC-Davis), under a separate but small project funded through the UC Salinity/Drainage Program.

Laboratory feeding studies will be conducted to examine the Se forms and their relationship to adverse effects in fish and their diet. Two resident fish species, splittail (*Pogonichthys macrolepidotus*) and bluegill (*Lepomis macrochirus*), will be employed. The life cycles of both species are amenable for laboratory studies of reproduction and embryonic development. In particular, this research team has successfully established the rearing and exposure facility for the Federally threatened splittail (CALFED project D113, #NFWF 99-07), which should greatly facilitate contaminant and physiological studies on this difficult species due to a shortage of its availability.

While a number of causes could contribute to the decline in splittail or other fish populations in the Bay-Delta, Se contamination is one that has not been investigated systematically. There are compelling reasons that Se impact on these fish species be explored: 1) the prime target for Se toxicity is reproduction, which is closely linked to population changes; 2) as a reactive metalloid and prooxidant, Se is prone to interact with other contaminants including heavy metals (e.g. Hg, Cd, Cu) and pesticides to inflict synergistic effects; 3) Se contamination in the Bay-Delta is bound to increase, in part due to the recently implemented agricultural drainage discharge upstream of the San Joaquin River.

Since Se toxicity of splittail is unknown, we will adopt bluegill as a model to facilitate studies on ecotoxic mechanism(s). Bluegill is a member of the *Centrarchid* family which is found to be generally more sensitive to Se effects. The devastating impact of Se on bluegill population has been well-documented in the Belews Lake incident (Lemly, 1985; Lemly, 1993) mentioned above. In addition, a comparison of these two species in terms of Se forms and adverse effects should facilitate the acquisition of "common" indicators for Se ecotoxicity.

Both fish species will be reared from the larval to reproductive stages in a partially closed recirculating system equipped with water pump, UV tube, biological and charcoal filters and fed with nutritionally balanced diets (Teh and Hinton 1998). This procedure assures adequate nutrition of known composition and gives excellent fish growth. The main diet will be composed of a purified casein-based diet (DeKoven et al 1992) plus brine shnmp nauplii (as larval fish diet) or adults (as adult fish diet); both diets will be prepared in the laboratory. Different Se-burden diets will be made from incubating brine shnmp cysts with commercial selenite yeast which contains up to 2000 ppm of Se in the biomass. We have analyzed one yeast batch for proteinaceous Se-Met which amounted to 960 ppm of the biomass. In addition, a preliminary growth trial of brine shrimp with the selenite yeast indicates that a wide range of Se-Met-containing diets (up to a few hundred ppm) *can* be prepared for the feeding studies. For comparison, field Se-laden brine shrimp will be collected from agricultural evaporation ponds, analyzed for Se forms, and mixed with the casein diet for parallel feeding studies.

Splittail and bluegill larvae (30 per replicate for three replicates) will be fed with diets containing proteinaceous Se-Met ranging from trace to 50 ppm with a high probability of reproductive impairment occurring in the higher Se diet treatments. Feeding schedules will include feeding with the same Se-laden diets continuously or in oscillation with low and high Se diets. The latter schedule may more closely resemble the field feeding conditions. A comparison of these two types of feeding schedules should help towards scheduling Se discharge limits to avoid BAC exceedance. Feeding will continue until individuals become gravid and 15 fish from each replicate will be necropsied. Gonad, liver, and muscle from individual fish will be collected and divided into two halves. The first half will be fixed in 10% buffered formalin and processed for histopathological analysis. Figure 2 illustrates an example of ovarian aberrations observed in fish species collected from Se contaminated waterways of the San Joaquin Valley.

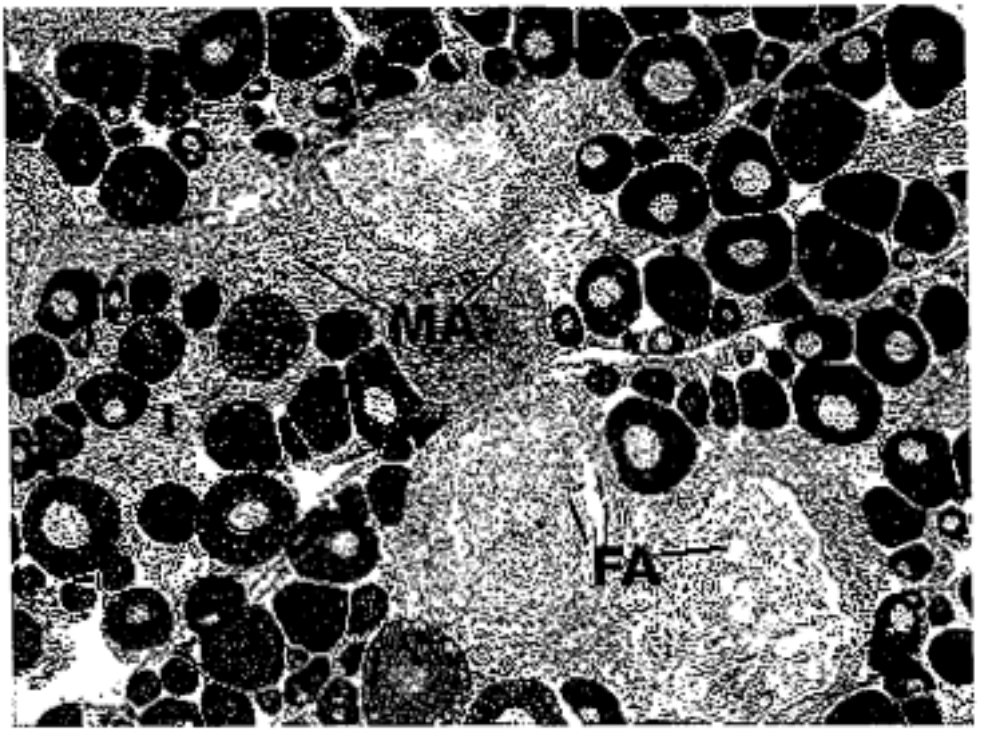


Figure **2.** Ovarian aberrations in carp collected from the San Luis Drain canal. **This** graph illustrates inflammation and cellular lesions in ovarian tissues of carp. **MA** = macrophage aggregate, **FA** = follicular atresia, I= inflammation.

The second half will be frozen in liquid N_2, lyophilized, and pulverized for Se analyses including total Se in biomass/protein-free and proteinaceous fractions, protein-free selenoamino acids, and proteinaceous Se-Met. The fish diets will also be subject to the same Se analyses. Total Se will be analyzed by the microdigestion/fluorescence method, free selenoamino acids by trichloroacetic acid (TCA) extraction, followed by MTBSTFA derivatization and GC-MS, and proteinaceous Se-Met by protein extraction, 6N HCI digestion, followed by MTBSTFA/GC-MS (Fan et al., 1998a & b). A proteinaceous Se-Met analysis of a deformed bird embryo by GC-MS is illustrated in Figure **3.**

For the developmental toxicity study, the remaining fish individuals from each exposure will be reared in separate tanks and allowed to spawn. Embryos will be collected and examined under a dissecting microscope for developmental dysfunctions. Intermittently, 15 embryos from each exposure group will be fixed in **10%** buffered formalin for histopathological analysis of developmental defects not detected by gross examination. If necessary, alterations in embryonic cells using enzyme-and immunochemical approaches will be examined (Teh and Hinton, 1993). The % of hatches, time to hatch, viability, and survival rates of embryos from each exposure will also be assessed. Moreover, cytotoxicity will be imaged using fluorescent molecular probes and confocal microscopy to determine cell viability and alterations in cytoskeleton. The cytotoxic evaluation is expected to yield a more sensitive indication of aberrations than morphological assessment by light microscopy. To demonstrate cell viability, propidium iodide exclusion will be used (Gagne and Blaise 1998). For cytoskeleton analysis, fluorophore-tagged antibodies against actin and cytokeratins (Henson et al., 1995) will be used to localize the cytoskeletal elements for determining whether changes have occurred in cell shape as a function of exposure to Se. After confocal microscopy, preparations will be removed and fixed by conventional methods for high-resolution light microscopy. Cellular imaging studies will then be compared with conventional high-resolution light microscopy for linkages between cellular/molecular events and histopathological lesions in fish. Combining the adult and embryo analyses, we anticipate to provide detailed information on fish toxicity at the molecular, cellular, tissue and organismal levels.

Aberrations in tissues and embryonic development will then be compared with the body and organ burdens of Se and Se concentration in diets to establish the Se threshold above which abnormalities begin to occur. The Se form(s) that best correlate with histological aberrations and cytotoxicity will also be identified and compared between the two fish species and their diets. These comparisons may help

reveal common a t o x i c indicators for the **two** species towards Se contamination. For example, it is possible that the propensity of Se-Met incorporation into proteins is positively correlated with Se toxicosis for both species. If so, these biochemical indicators developed by the proposed project may be applicable to a wide range of fish species for **risk** assessment,

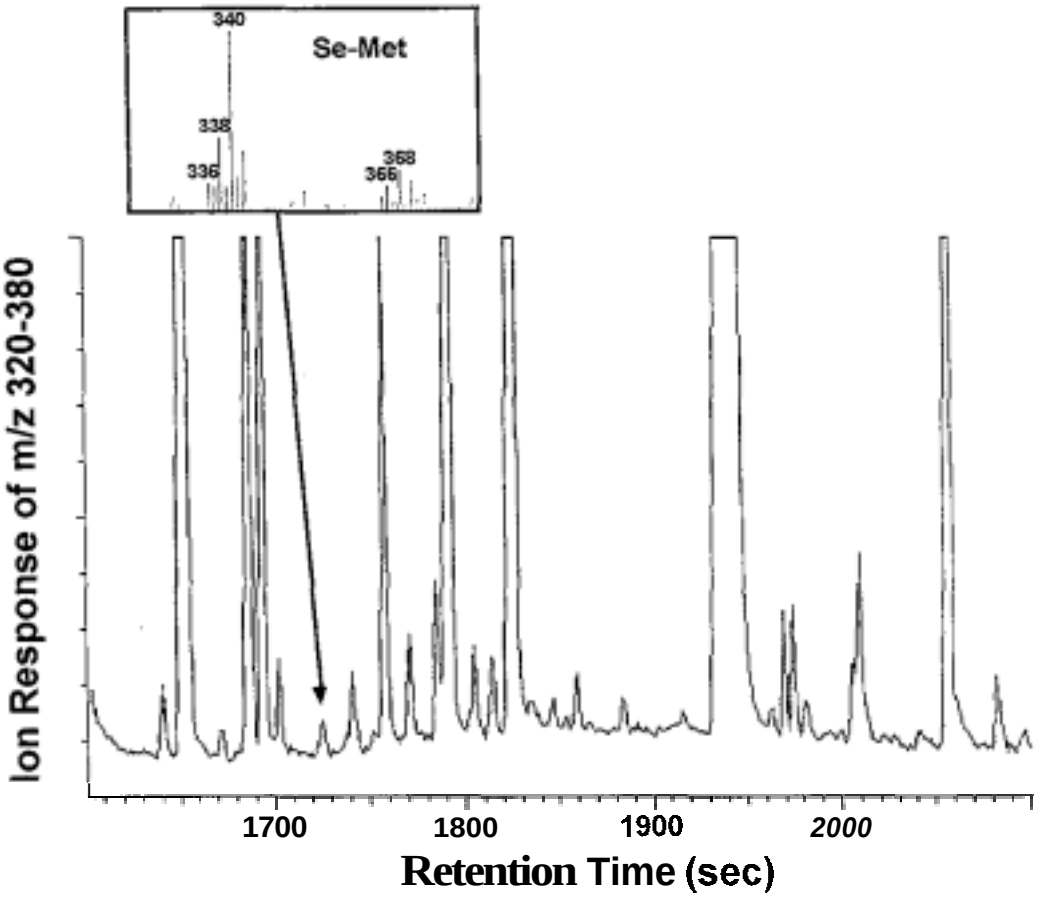


Figure 3. Proteinaceous Se-Met analysis of a deformed bird embryo. The stilt embryo was collected from a Se-laden site in the San Joaquin watershed. The embryo was lyophilized, pulverized, and extracted for proteins according to Fan et al. (1998a). The protein extract was digested in 6 N HCl to release amino acids which was then silyllated with MTBSTFA before GC-MS analysis using a narrow range scan (m/z range of 320-380) mode. Se-Met was identified based on the mass fragmentation pattern and GC retention time by comparison with the standard. We are improving the sensitivity for Se-Met analysis by using the selective ion mode, which has recently achieved analysis of fish samples containing background level of Se-Met.

Objective 2: Utilize results from Objective 1 to uncover biochemical forms of Se with the potential to be assa ed co venientl which

The experimental approach in this section of the proposal will depend on the outcome of the research for Objective 1. For example, if protein-free selenoamino acids are the main form(s) associated with ***Se*** toxicity, then the TCA extraction/GC-MS method developed from Objective 1 *can* be directly employed for field application (Objective 3). However, the more likely outcome would be that proteinaceous Se or Se-Met is best correlated with the histological and cellular aberrations, based on previous observations (see section B2).

If so, the protein extracts obtained from Objective 1 will be further separated into individual seleno-proteins. This route of investigation *can* be important since specific selenoproteins might represent more reliable biochemical indicators of BAC exceedance than the total proteinaceous Se or proteinaceous Se-amino acids. The protein extracts will be analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the fractionated proteins analyzed for total Se. Once the subunit molecular weights of the seleno-proteins are determined from SDS-PAGE, the same protein extract will be fractionated by the size-exclusion HPLC method so that a larger amount of the selenc–proteins *can* be collected. The HPLC fraction will then be checked for protein purity by 2-D gel electrophoresis with isoelectrofocusing (IEF) separation in the 1st dimension, followed by SDS-PAGE for the 2nd dimension. Figure **4** illustrates an example 2-D electrophoresis result of a metallothionein extract of Asian clam (*Portamocorbula amurensis*) collected from the Bay-Delta region showing Cd contamination by Dr. San Luoma and his group at USGS, Menlo Park

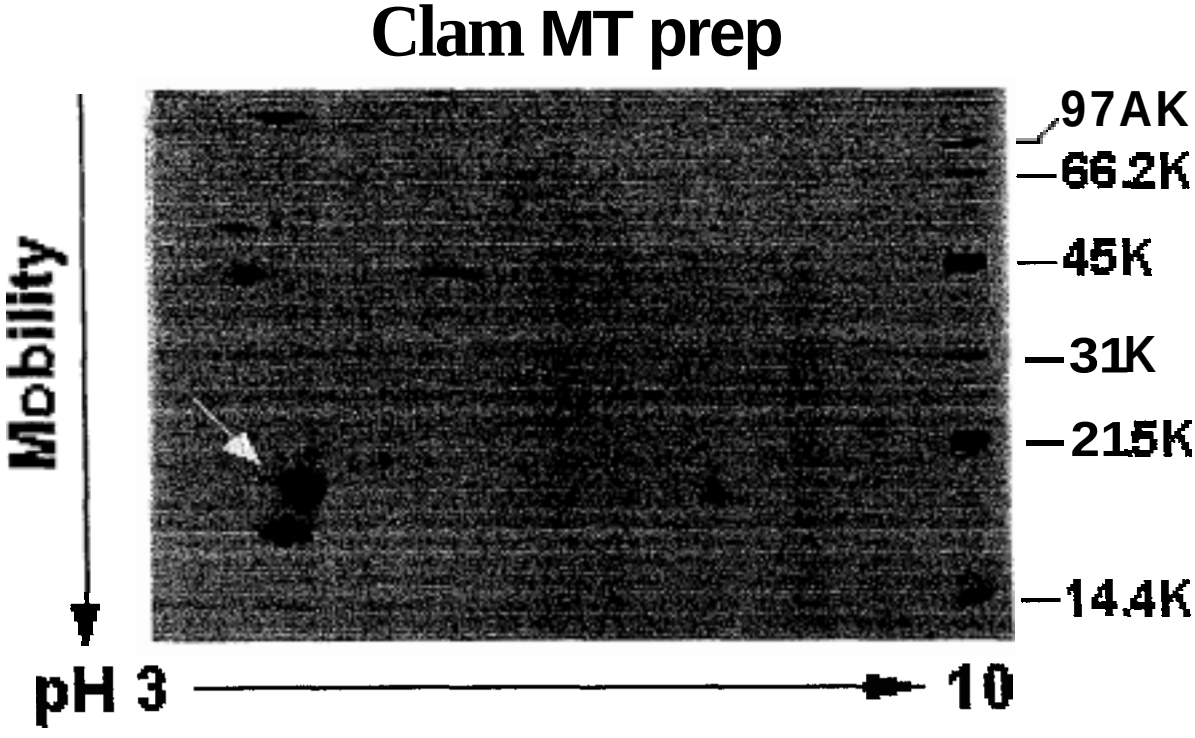


Figure 4. 2-D gel electrophoresis of a metallothionein preparation *of* Asian clam collected from the San Francisco Bay/Delta. The clam was extracted for small proteins and peptides with a buffer containing 50% acetonitrile. The extract was then subject to IEF (pH **3-10)** separation in the 1st dimension, followed by SDS-PAGE in the 2nd dimension. The proteins in the gel was visualized with silver staining and the arrow indicates the protein spot that corresponded to metallothionein. From such 2-D separation, it should be feasible to obtain highly purified proteins of interest.

Through such 2-D PAGE analysis, the protein purity *can* be examined and highly purified seleno-proteins *can* be obtained for subsequent characterization (e.g. analyzed for Se-Met and other amino acid content as described above). Although the proteins *can* be subject to peptide mapping by LC-tandem MS in an attempt to identify the protein from a database, this is beyond the scope of the proposed project. If time and resources permit, the purified proteins that relate to toxicity– that is, the candidate indicators of BAC exceedance - will be used to produce antibodies for convenient immunochemical assay. Alternatively, the protein *can* be sequenced for amino acids, from which appropriate primers *can* be developed for amplification of cDNA probe by polymerase chain reaction (PCR). These molecular tools can then be used for investigations under Objective *3*.

Objective 3: Test these indicators in field studies. with the aim of assessing exceedance of biological assimilatory capacity on a site-specific basis.

The information from the first two Objectives will be applied to the analysis of the same fish species collected from selected Se-contaminated sites. These sites will be located in the San Luis Drain (SLD) agricultural drainage canal and its receiving waters, the San Joaquin river *(SJR)* and its confluent San Francisco Bay-Delta. Bluegill is regularly found in SLD and SJR while splittail dwells in the Bay-Delta. The San Luis Drain has been in use since 1997 for the discharge of Se-laden agricultural drainage from the western side of the San Joaquin Valley, CA. There is a current EPA-mandated Se load limit (which amounts to about 6000 pounds/year) that *can* be discharged into the San Joaquin river from the drain canal. This load limit was set based on the historical Se load calculation, but the actual impact on the receiving waterways has not been assessed.

Other than input from the San Joaquin River, the Bay/Delta gets additional Se discharges from oil refinery facilities. Although population-level changes in certain fish and avian species (e.g. splittail, sturgeon, diving duck) have been reported (US Fish & Wildlife Services, 1995a&b), it is highly controversial whether Se contamination may have contributed to these changes. This is because the two connecting waterways also receive a number of other pollutants from point and non-point sources including transition metals, Hg, pesticides, PAHs, and PCBs. The Se-specific effect indicators to be developed should help towards resolving the controversy.

Fish will be captured by electroshocking or netting during spring seasons and gonad, liver, and muscle tissues will be collected on-site. Half of the tissues will be preserved in 10% buffered formalin for histological examination while the other half will be frozen in liquid N_2 and transported on dry ice back to the laboratory. For splittail, tissue samples will be provided from the CALFED splittail project D113, also on which Dr. Teh is co-PI. These tissues (bluegill and splittail) will be processed and analyzed for histological and cellular aberrations, total Se, and Se forms as described in Objective 1. Analysis of variance (ANOVA) will be used to compare the Se and histological/cellular results, which will then be correlated with those from the laboratory feeding studies. The field results should help validate the laboratory findings in terms of the reliability of the identified indicators for Se risk assessment in the Bay-Delta watershed.

If time permits, the antibody or cDNA probes will be tested on field samples for toxicity-Elated selenoprotein(s) using ELISA or blot hybridization method. If successful, these fast-screening tools should greatly facilitate analysis of Se-specific indicator(s) for BAC exceedance.

C.2.c. Monitoring and Assessment Plans

This section is not applicable since this project does not involve an implementation or a pilot/demonstration.

C.2.d. Data Handling and Storage

GC-MS analysis data will be reduced using the Hewlett-Packard Chemstation or Finnegan TrapMaster software. Peak areas will be integrated and converted to a spreadsheet file. NMR data will be reduced using NUTS software, and fluorescence spectra acquired and analyzed using Perkin-Elmer FL Winlab software. HPLC data will be acquired and reduced using Peak3 chromatography software. Both histopathological and PAGE images will be recorded using high resolution digital imaging systems. In general, individual analyses will not be immediately repeated; instead, entire experiment sets will be repeated to confirm the overall results, since the trends in the results is the purpose of the studies. Thus, the emphasis throughout the study will be on accuracy of the trends, and not on the precision of the data. As such, it is not practical at this time to state the statistical tests that will be used for most data. Standard laboratory data-logging practices such as page-numbered notebooks and entries in ink will be followed. The majority of - and most important - information from this project will consist of digital data acquired by instruments, or the result of computation using the raw data We use a multi-tier system of data

backup in which the primary data is immediately copied to another computer (via FTP or 100Mb cartridges), from which additional backup onto 650Mb CD-ROMs are performed.

C.2.e. Expected Products/Outcomes

The main products of this research will be information, measurement techniques, and knowledge regarding exceedance of BAC. The physical products will be reports to CALFED and submitted to a high quality scientificjournals for peer review and publication. Results will also be disseminated widely through participation in workshops and seminars, and presentation of papers at international and national scientific meetings.

We antidpate the BAC exceedance in bluegill and splittail from field and laboratory studies will correlate with changes at the ecological level (DFG and IEP ongoing studies), and that it will complement the extensive water, tissue, and sediment analyses for contaminants (SFEI/CMARP and USGS ongoing studies). In total, the output products of all these studies will provide a fundamental understanding of both the ecotoxic (foodchain-mediated)and toxic (organismal) mechanism(s) that underlie the teratogenic effects and reproductive failure observed for Se-inflicted top predators, and will pioneer an approach that *can* generally be applied to other aquatic ecosystems.

C.2.f. Work Schedule

The two Tasks (analysis for Se forms and histopathology) to fullfill the three Objectives run the full duration of the project, 3 years. The proposed schedule for the work plan is as follows. Fish rearing and feeding of Se diets will commence immediately and be performed throughout the first two years. Histology/cellular imaging and biochemical analyses will begin when fish reach reproductive stage and be conducted throughout the first two and half years. Field sample collection and analyses will begin during the first spring season of the project and be conducted in each subsequent spring season.

C.2.g. Feasibility

The proposed workplan is highly feasible since the heart of the proposed tasks of Se form analysis as well as fish rearing and feeding, and histolopathological/cellular assessment has been established by this team (see c.2. Proposed Scope of Work). All types of analyses to be performed is currently performed in the PIs' laboratories, and all Se analysis methods were developed by the PIs. These facts ensure a thorough understanding of the analyses, limitations, and quality assurance aspects by the research team. Dr. Teh has established vertebrate (fish) protocols according to University guidelines (protocol #8937, Feb 2000), and in fact has a culture of splittail for research purposes. In general, the detailed mechanistic studies in the laboratory to establish relevant Se ectoxic indicators for splittail (or any other fish species) is aimed at reducing the number of field samples needed for meaningful assessment. This is of particular importance to species of reduced or threatened populations.

Field collections of fish are feasible, as it will be performed by Dr. Mary Dunne and her team of the California Dept. of Fish and Game, under our existing collaboration. For the CALFED project, should it be funded, we will re-evaluate the choices of collection sites to optimize the project Objectives.

For the longer term, beyond the scope of the proposed project, the main uncertainty is whether a quick and convenient assay for specific selenoprotein(s) that are indicative of BAC exceedance *can* be devised, since little is known about the nature of these proteins in aquatic fish and wildlife in general. Of course, this lack of knowledge is the very reason for this proposed investigation. However, based on the lesson learned from mammalian systems, only a handful of major selenoproteins are present, instead of spreading into a large number. This should greatly reduce the difficulty in detecting and characterizing these proteins.

D. APPLICABILITY TO CALFED ERP GOALS

D.1. ERP GOALS AND CVPIA PRIORITIES

The proposed research should help attain at least two of the ERP goals, i.e. Goal 1 – At-Risk Species; Goal 6 – Sediment and Water Quality. By addressing the key scientific uncertainty associated with Se contamination and impact on at-risk fish in the Delta and sensitive species in the SJR, this research should help identify Se sources and ecotoxic impact to the Bay-Delta fish populations.

The current knowledge base regarding selenium is inadequate. It is well-known (e.g. EPA, 1996; EPA Office of Water, 1998) that the present dependence on waterborne selenium concentration is NOT a reliable indicator of downstream biological impacts. A leading scientist in this area recently stated that *"...measures of waterborne selenium alone would be inadequate for assesssing toxic* **risk.**" (Skorupa, 1998). According to a CALFED document (CALFED–306, 1999), *"A question has been raised over the adequacy of concentration-based standards.... EPA has convened a nine-member panel in a Peer-Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation that is investigating the need for differentiating the toxicity of different forms of selenium and developing site-specific objectives for selenium."* The principal investigator of this proposal, Dr. Teresa Fan, is a member of this EPA panel that is defining this shift from the current waterborne Se concentration standards to bioindicators of greater relevance to ERP goals. The toxicity mechanism-based risk indicator(s) to be determined from this research should help guide other restoration efforts, in particular to help avoid ecosystem conditions that further aggravate Se impact.

Similarly, the project directly addresses the major CVPIA "Biological Principles", because the mechanistic understanding and the assessment tools developed by the proposed study for estimating selenium impacts will be applicable to all fish species. Folding the biological impact mechanism(s) into the concept of exceedance of Biological Assimilatory Capacity (BAC) extends applicability to ecosystem levels. Moreover, the reliable and convenient BAC exceedance indicators will assist with management or remediation efforts associated with Se discharge into the Bay-Delta.

D.2. RELATIONSHIP TO OTHER ECOSYSTEM RESTORATION PROJECTS

The work proposed here bears a special relationship to CALFED project 99-D113, "Chronic Toxicity of Environmental contaminants in Sacramento Splittail (Pogonichthys macrolepidotus): A Biomarker Approach", because fish from the splittail culture established by that project will be utilized here. Dr. S.J. Teh is a co-PI on both projects. Please refer to section D.4 below for a summary of D113.

In addition, the studies from this project may provide valuable additional interpretability to the results from CALFED project F1-106, "Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary" by W.A. Bennett, S.J. Teh, and S.L. Anderson. Although that project has ended, Dr. Teh, who performed the histopathology for F1-106, is also co-PI on this proposed project, providing an opportunity for additional interpretation in light of the proposed mechanistic studies. A summary of F1-106 is found in section D.4 below.

In many ways, the proposed project is highly complementary to the studies being performed in CALFED project F1-103, "Assessing Impacts of Selenium on Restoration of the San Francisco Bay-Delta Ecosystem" by S.N. Luoma, G.A. Cutter, N.S. Fisher, and D.E. Hinton. F1-103 is investigating bioavailability relationships of Se thru the lower foodchain to sturgeon, while this project will investigate the biochemical forms of Se involved in foodchain transfer and relate it to sensitive histopathological biomarkers. Thus, the results of this project, together with F1-103 of Luoma et al. should provide a more complete picture of the Se biogeochemistry and sensitive biochemical and biomarkers of impacts.

The development of measures of biological assimilatory capacity (BAC) exceedance – the main task in this proposal – should represent the next generation of adaptive management, using the framework

pioneered by CALFED projects such as F1-252, "San Joaquin River Real-Time Water Quality Management Program" by E.W. Cummings, J.A. Kipps, L. Grober, and N. Quinn, and its complementary CALFED project 99-D100, "Real-time Water Quality Management- San Joaquin River". Logically, the exceedance of biological assimilatory capacity would be a more relevant measure of biological impacts than the physical-chemical assimilatory capacity currently used. However, BAC exceedance cannot be implemented at present **because** its parameters are currently unknown; this proposal is specifically designed to obtain this information.

The measures of BAC exceedance should also greatly assist ongoing remediation studies, such as CALFED project F1-273, "Imgation Drainage Water Treatment for Selenium Removal: Panoche Drainage District Demonstration Facility" by W.J. Oswald. In this case, the measures of BAC exceedance, once developed, may be used as a biologically-responsive criteria for adjusting Se treatment parameters and rate of discharge; the latter would probably be tied into the real-time management system mentioned above.

D.3. REQUESTS FOR NEXT-PHASE FUNDING

This section is not applicable to this proposal.

D.4. PREVIOUS RECIPIENTS OF CALFED FUNDING

CALFED F1-106: Role of Contaminants in the Decline of Delta Smelt in the Sacramento-San Joaquin Estuary (Agreement No. B81650).

The goal of the project was to evaluate the overall health, condition, and growth rate of delta smelt collected from various habitats encompassed by the Interagency Ecological Program (IEP) monitoring surveys. Our investigation of these samples employs evaluation of: 1) histopathology biomarkers of exposure and organ/tissue condition, 2) biomarkers of DNA damage, and **3)** otolith growth rate analyses of individual smelt Integration of these state-of-the-science techniques will quantify potential contaminant effects on individuals that ***can*** be related to consequences for the delta smelt population. Dr. William Bennett (PI) has developed methodology and completed surgery to remove otoliths on over 400 delta smelt specimens. Growth rate has been evaluated on over 50 specimens. Dr. Swee Teh has finished processing and evaluating 400 smelt specimens. He has submitted the standard operating procedure and histopathology results to the PI. Quarter 1 progress report had been submitted to the CALFED. The investigators are currently generating the Annual progress report for CALFED.

CALFED *D113: Chronic Toxicity of Environmental contaminants in Sacramento Splittail (Pogonichthys macrolepidotus): A Biomarker Approach (Agreement No. NFWF99-N07).*

This project integrates field and laboratory studies to determine chronic toxicity to splittails, a federally threatened species. This biomarker study will be performed in conjunction with ongoing efforts by Department of Fish and Game, San Francisco Estuary Institute and US Geological Survey. We have only recently received funding for this project, but the investigators (Teh and Hung) have already successfully spawned a large number of splittail under laboratory conditions. We have finished a splittail growth study and have determined the proper laboratory diets for splittail. In addition, We have also finished a pilot study of exposing 2 day-old embryo to aqueous sodium selenite. A Quarterly progress report will be submitted to CALFED on July 15, 2000.

D.5. SYSTEM-WIDE ECOSYSTEM BENEFITS

Currently, there is no funded project addressing the need for early-warning indicators of environmental deterioration in a given watershed. This lack is particularly outstanding for pollutants such as Se that exert its impact through extensive biogeochemistry, resulting in unreliable correlations **between** chemical concentration and bioeffects. This project, by establishing measures of BAC exceedance of Se, will be highly complementary with ongoing biomonitoring efforts of fish population by DFG and USFWS and water, sediment, and tissue contaminant monitoring by USGS and CMARP. Measures of BAC exceedance will provide valuable information for future environmental compliance

and regulatory studies and the ecological risk assessment process, eventually helping to guide management decisions on determining acceptable contaminant levels in the environment

This project will also support ongoing efforts by the USFW Service and the IEP in recovering threatened and endangered fish populations in the Sacramento-San Joaquin system by facilitating restoration planning and monitoring. We will be working in close collaboration with DFG's splittail monitoring survey, IEP funded splittail culturing projects, CALFED funded splittail project (#D113), and various water quality monitoring programs, such as the "Real-time"water quality monitoring programs for selenium which are CALFED projects F1-252 and 99-D100.

E. QUALIFICATIONS

Brief Profiles of Investigators

Dr. Teresa** W-M. Fan* is a faculty research environmental biochemist in the Department of Land, Air and Water Resources, University of California, Davis. Her research interest has been in the broad area of environmental biochemistry ranging from plant stress biochemistry and Se biogeochemistry in relation to ***in situ bioremediation, to mechanisms of aquatic ecotoxicity of agricultural and industrial discharges. Along CalFed's interest, she has been working on salinity and toxic metals stress on the Asian clam, *Potamocorbula amurensis*, in the Delta/San Pablo Bay, as well as the tradeoffs between algal phytoremediation and ecotoxic risk of selenium in San Joaquin Valley's evaporation ponds. She has served on the 9-member EPA Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation (see EPA Office of Water, 1998) which concluded that selenium organic forms and foodchain biochemistry - not total Se - should be the target of ecotoxic investigations and bioremediation goal. Most recently, she was one of the authors of the Central Valley Drainage Implementation Program's comprehensive report on Discharge to the San Joaquin River.

Dr.** Richard M. Higashi* is a faculty research environmental chemist in the Crocker Nuclear Laboratory, University of California, Davis. He has worked in broad areas ***of environmental chemistry, ranging from toxicity identification in complex effluents such as pulpmill and oil production discharges, to DOE waste contamination remediation, to agricultural water, soil, and sediment problems of the Central Valley and San Francisco Bay/Delta, as well as air pollution (PM10 and ozone) research in the Central Valley and Sierra Nevada Range. The chemistry of humics and other organic matter plays a central role in ALL of these research areas, and he is currently engaged in organic matter chemistry investigations in relation to selenium ecotoxic remediation in evaporation ponds of the SJV.

Dr.** Swee J. Teh* is a comparative pathologist with 14 years of extensive field and laboratory research experience on ecotoxicology and biomarker study. He has also experienced in projects and experimental design and managing contracts and grants' total >$1 millions per year. He will be primarily responsible for 1) the histopathological and histochemical assessment of fish obtained from the field; 2) the histopathological and histochemical assessment of control fish exposed to chemical mixtures in the laboratory, 3) the interpretation and integration of all histopathological and histochemical data; and 4) the submission of quarterly and annual reports with Drs. Fan and Higashi. He has been Principle Investigator and/or managed grants from various Federal agencies, including USEPA, NCI, and CALFED. Dr. Teh is the primary or co-author on over two dozens referred publications related to invertebrate and fish histopathology, histochemistry, and ecotoxicology, including Teh and Hinton (1993 and 1998) and Teh ***et al. (1997 and 1999).

Responsibilities. Dr. Fan will assume the responsibility of overall project management and the biochemical part of project including training on sample handling, Se analyses, and gel electrophoresis. Dr. Higashi will be responsible for the GC-MS, HPLC, and LC-tandem MS development for Se form separation and analyses. One postgraduate researcher (PGR V) will work with Drs. Fan and Higashi in conducting the bulk of the Se analyses, gel electrophoresis, and HPLC separation. Dr. Teh will oversee the fish rearing, histopathology, and cellular imaging part of the project and supervise experimental design and interpretation of data Dr. Teh will be responsible for training and coordinating technician

and graduate student for these tasks. The named technician (Foo-Ching Teh) will perform processing, sectioning, and staining of all fixed tissues intended for histopathologic or histochemical assessment One graduate student will conduct fish rearing and feeding studies, as well as assists Dr. Teh in cellular imaging. All personnel will participate in the design of feeding studies and data interpretation.

Facilities

Drs. Teresa Fan and Richard Higashi's laboratories are jointly equipped with a Varian 3400 cryogenic-capable capillary GC interfaced to a CI-equipped Finnegan ITD 806 mass spectrometer (MS), and two HP5890 GC each interfaced to a HP5971A MSD, these three GCMS systems are not shared instruments and are dedicated to the PIs' research. *One HP* GC-MSD system is interfaced to a CDS Pyroprobe 2500 Autosampler Analytical Pyrolysis instrument for dedicated pyro-GCMS analysis while the other is connected to a HP 7673 liquid autosampler. Analysis of semipolar and polar organics is routinely performed on these GC-MS systems, including pesticides, PAH metabolites, PCBs, resin acids, organic acids, amino acids, sugars, and fatty acids, as well as Se metabolites; also frequently analyzed are a wide range of substances from sulfur/selenium gases to insoluble macromolecules (e.g. humics and lignin). In addition, dedicated to element analysis is a Jordan Valley EX3600 ED-XRF spectrometer modified with narrow collimated beam for small samples. There are two non-shared HPLCs equipped with UV-Vis, fluorescence detectors, metal-free Timberline RDR-1 post-column reactor, and Alltech Evaporative Light Scattering Detector; these systems are used primarily for peptidelprotein and inorganic ions analyses. Other non-shared equipment are: a new Perkin-Elmer LS-50B scanning spectrofluorimeter capable of rapid acquisition of excitation-emission matrix and X-Y positioning stage w/ fiber optics for 96-well plate/gel/filter reading, Precision Detectors PDDLS/Batch photon correlation spectrometer for macromolecule/colloid sizing, Hewlett-Packard 5483 diodearray UV-vis-NIR spectrophotometer, Owl Scientific, CBS, Bio-Rad, and ATTO 2-D gel electrophoresis systems and Owl Western Blot apparatus, and an Olympus BH2 Hoffman Modulation Optics microcroscope with epifluorescence. Shared with other PIs include a Perkin-Elmer AutoImage/System 2000 FTIR microspectrometer capable of chemical imaging spectroscopy at 10 μm resolution.

The proposed research on foodchain and cellular imaging will be carried out in School of Veterinary Medicine laboratories and the Aquatic Toxicology Exposure Facility (ATEF) of Drs. Hinton and Teh. The ATEF is a 3,200 ft^2 facility for aquatic toxicologic investigations, which include studies using US EPA three species tests and exposure of fish and invertebrates to numerous toxicants. A 240 ft^2 high containment exposure laboratory (HCEL) has dedicated exhaust, equipment for bulk storage, weighing and dilution of volatile toxicants, and aquaria for exposure of fish under continuous flow conditions. The HCEL is reached by passage through a 42 ft^2 safety alcove and a larger (800 f?) intermediate exposure room (IER) with dedicated exhaust. A 1,600 ft^2 "wet lab" is equipped with waterproof lighting and electrical outlets. A variety of different types of environmental conditions may be mimicked in "growout" modules. Fish will be maintained at a ATEF facility that is a 1500 sq. ft. metal quonset hut and a 800 sq. ft. indoor preparation laboratory. There are 72 small (90 liter) and 6 large (700 liter) fiberglass tank systems in the 1500 sq ft. quonset hut and an outdoor system with 15 large (700 liter) fiberglass tanks supplied with 400 liter/min of 19 ± 1°C aerated well water all year round. This facility is an AALAC-approved facility.

The preparation laboratory is equipped with scales, refrigerators, freezers, and instruments needed for fish rearing and biological sample preparation. Laboratory and university personnel monitor these facilities 24 hours daily. Purified and Test diets, and its chemical and toxicant contents, will be prepared and verified in the laboratory which is equipped with several feed mixers, a steam generator connected to a California Laboratory Pellet Mill, HPLC, GLC, spectrophotometer, and atomic absorption. Biochemical and cellular imaging studies will be performed in 1203 Haring Hall, which is a 200 ft^2 general purpose laboratory for necropsy, processing, microscopy, histology, stereology and histochemistry lab for biochemical and molecular toxicology. It is equipped with Pearse tissue freeze drier, FTS tissue-dry; 6 fthood; pH meter, balances, computer-assisted morphometry workstation, LKB historange microtome, HistoStat frozen section microtome, 2 ultramicrotomes; support equipment for holding fish for necropsy; PM10AD photographic camera system; Olympus SZH dissecting photomicroscope, Olympus BH 2 research binocular microscope with phase (Nomarski), wide, flat field optics: All attachments for fluorescence are included. B&L model 1201 UV-visible spectrophotometer; a

Brinkman model RMS refrigerated water circulator, an Orion model EA 920 expandable ion analyzer, a Fisher model 348 G refrigerator, Revco -80°C freezer, 2 sinks, hot, cold and distilled water sources. Fisher histomatic slide stainer model 172; 820 Spencer microtome for paraffin; Tissue Tek II Tissue embedding. The Anatomy Department houses a Bio-RAD MRC 1024 ES Confocal Microscope.

F. COST

Federal budget forms (SF-424A) are presented at the back of the proposal package, as per instructions. That budget is identical to the one presented below, $651,288, which is based on California State Resources Agency 10% overhead. Other agencies may be higher. For example, the current overhead rates for Federal agencies are 46.5, 48.0, and 48.5% for years 1, 2, and 3, respectively. In this case, the total budget will be $871,559; both the "State" and "Federal" budget totals are also stated on the proposal cover page.

F.1. BUDGET JUSTIFICATION

Dr. Teresa Fan will serve as the lead-PI, who together with Richard Higashi will serve as co-directors of the "Se Forms" component of the project. The labor hours (20%) reflect the commitment of these co-PIS on method development and advanced analysis of selenium biochemical forms. A full-time postdoctoral associate is requested to help conduct experiments, process samples, and handle the principal analytical load of analysis for selenium forms. The supplies budget reflects common laboratory equipment, gel supplies, GC-MS maintenance, HPLC columns and solvents, and other items needed for conducting the Se forms analysis. Miscellaneous costs include travel for presentations ai national scientific meetings.

Dr. Swee Teh will serve as a Co-PI directing the "Fish Histopathology" component of the project. Direct labor hours reflect the commitment of Dr. The (25%) on these and his primary task of evaluating and interpreting the large number of histopathological preparations. Dr. Teh requires two assistants, a laboratory Assistant IV (C. Teh, 50%) for histological processing, as well as a Post-doctoral researcher (Dr. DongFang Deng, 50%) who specializes in fish husbandry and nutrition, and will be responsible for overseeing the preparation of contaminant-laden test diets. Dr. Deng and Teh need basic desktop computer and a compound light microscope for their dedicated use. Miscellaneous costs include supply funds for histology, histochemistry, laboratory exposures, computer software, and general laboratory/ office operation related to the project. In addition, these cost include travel funds for research, project meetings and presentations at the national scientific meetings.

There are no "Project Management" costs *per se*, as this task is integral with the research tasks. In the tables below, 10% overhead is calculated on direct costs minus equipment. Indirect costs, 10%, are based on funds from California State Resources Agency. The overhead rates for Federal agencies are 46.5, 48.0, and 48.5% for years 1, 2, and 3, respectively, yielding atotal budget of $871,559.

F.2. COST-SHARING

Although there is no direct cost-sharing of funds *per se*, this project will have synergistic, "value-added" fiscal advantages through interaction with other ongoing projects. In particular, the work proposed here bears a special relationship to CALFED project 99-D113, "Chronic Toxicity of Environmental contaminants in Sacramento Splittail (Pogonichthys macrolepidotus): A Biomarker Approach", because fish from the splittail culture established by that project will be utilized here. Hence, the costs of the raising the splittail stock and development of exposure systems is borne by that project.

The PIs also have projects on selenium ecotoxic risk, working ai the lower trophic and sediment chemistry levels, from the Univ. of California Salinity/Drainage Program and California DWR ("Microphyte-mediated Se Biogeochemistry and Its Role in Bioremediation of Se Ecotoxic Consequences, T.W. Fan, PI; "Chemical Nature of Selenium in Agricultural Drainage Sediments and its Implications for Bioavailability", R.M. Higashi, PI). Thus, the cost of development of Se biochemical techniques are partially borne by such projects. The costs of over $200,000 in analytical instrumentation required for the proposed research was entirely borne by grants from agencies such as USEPA, USDOE, and the EPA/UC-Davis Center for Ecological Health Research.

TOTAL BUDGET SUMMARY

Task	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acquisition costs	Miscellaneous & Other Direct costs	Overhead & Indirect costs	Total Costs
#1 Se Forms	8,064	251,837	0	39,000	6,000	29,685	326,522
#2 Fish Histopathology	7,200	227,768	0	65,245	3,000	28,753	324,766
Project Management	0	0	0	0	0	0	0
TOTALS	15,264	479,605	0	104,245	9,000	58,438	651,288

QUARTERLY BUDGET

Task	Year 1 Sep 00-Nov 00 (includes any Equipment)	Dec 00 - Feb 01	Mar 01 - May 01	Jun 01 - Aug 01	
#1 Se Forms	26,455	26,455	26,455	26,455	
#2 Fish Histopathology	33,423	24,922	24,922	24,922	
Project Management	0	0	0	*0*	
TOTALS	59,878	51,377	51,377	51,377	
	Year 2 **Sep 01-Nov 01 (includes any Equipment)**	**Dec 01 - Feb 02**	**Mar 02 - May 02**	**Jun 02 - Aug 02**	
#1 Se Forms	26,911	26,911	26,911	26,911	
#2 Fish Histopathology	26,329	26,329	26,329	26,329	
Project Management	0	0	0	0	
TOTALS	53,240	53,240	53,240	53,240	
	Year 3 **Sep 02-Nov 02 (includes any Equipment)**	**Dec 02 - Feb 03**	**Mar 03 - May 03**	**Jun 03 - Aug 03**	**GRAND TOTAL**
#1 Se Forms	28,266	28,266	28,266	28,266	326,522
#2 Fish Histopathology	27,815	27,815	27,815	27,815	324,766
Project Management	0	*0*	*0*	0	*0*
TOTALS	56,080	56,080	56,080	56,080	651,288

G. LOCAL INVOLVEMENT

This is a research project where the majority of the work will be done within research laboratories at Univ. of California, Davis. Therefore, local, environmental, landowner, conservancies and CRMPS, groups are not affected by this project. Thus, there is no public outreach planned, except for reports to CALFED and publications in the peer-reviewed scientific literature.

H. COMPLIANCE WITH STANDARD TERMS AND CONDITIONS

Please see on the following page the letter addressing the Univ. of California, Davis position.

UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

srndowdy@ucdavis.edu
OFFICE OF THE VICE CHANCELLOR FOR RESEARCH
(530) 752-2075
FAX (530) 752-5432

410 Mrak Hall, One Shields Avenue
DAVIS, CALIFORNIA 956168671

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, CA 95814

MAY 1 1 [illegible]

Dear Colleague:

2001 Proposal Solicitation
Proposal Entitled "Evaluation of Biological Assimilatory Capacity for Mechanism-Based Adaptive Management for Selenium in the San Francisco Bay-Delta"
Principal Investigator: Teresa W-M Fan

It is a pleasure to present for your consideration the referenced proposal

Following the direction of "Attachment **D** - Terms and Conditions **for** State Proposition **204** Funds", this is to provide notification that the applicant takes exception to the following proposed "standard" clauses:

Section 6. Substitution
Section 9. Rights in Data
Section 11. Indemnification, and
Standard Clauses-Insurance Requirements - DWR

In order to bring the above provisions into conformity with the University of California Policy, we reserve the right to discuss with the aim of properly modifying these sections, should this proposal result in a subsequent award.

Please contact the principal investigator for scientific information. Administrative questions may be directed to my assistant, Ms. Petrina Ho, or me by telephone, facsimile or electronic mail at the numbers cited above. Furthermore, correspondence pertaining to this proposal and any subsequent award should be sent to the Office of Research and to the principal investigator.

Sincerely

Sandra M. Dowdy
Contracts & Grants Analyst

Enclosures
Cc: T. Fan

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J. THRESHOLD REQUIREMENTS

The State and Federal contract forms, including the Federal budget forms, Environmental Compliance Checklist, and Land Use Checklist are on the following pages. There are no letters of notification, since this is a research project that does "not include any physical action on the ground", as stated in the CALFED 2001 PSP, p. 50.

APPLICATION FOR FEDERAL ASSISTANCE

OMB Approval No. 0348-0043

2 DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	State Application Identifier
4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

1. TYPE OF SUBMISSION:

Application: ☐ Construction ☒ Non-Construction

Preapplication: ☐ Construction ☐ Non-Construction

5. APPUCANTINFORMATION

Legal Name: Regents of the University of California

Organizational Unit: Dept. of Land, Air and Water Resources

Address *(give city, county, State, and zip code):* Office of the Vice Chancellorfor Research, 410 Mrak Hall Univ. of California, Davis, One Shields Ave. Davis, CA 9561**6** Yolo County

Name and telephone number ofperson to be wntacted Mlmatters involving this application *(give area code)*: Teresa W-M. Fan, 530/752-1450

6. EMPLOYER IDENTIFICATIONNUMBER *(EIN):* 94 – 6036494 W

7. TYPE OF APPLICANT: *(enter appropriate letter in box)* I

A State — H. Independent School Dist.
B. County — I. Stale Controlled Institution of Higher Learning
C. Municipal — J. Private University
D. Township — K. Indian Tribe
E. Interstate — L. Individual
F. Intermunicipal — M. Profit Organization
G. Special District — N. Other (Specify) _____

8. TYPE OF APPUCATION

☒ New ☐ Continuation ☐ Revision

If Revision, enter appropriate letter(s) in box(es) ☐ ☐

A. Increase Award B. Decrease Award C. Increase Duration
O. Decrease Duration Other *(specify):*

9. NAME OF FEDERAL AGENCY: CALFED Bay-Delta Program

10. CATALOG **OF** FEDERAL **DOMESTIC** ASSISTANCE NUMBER ☐☐ – ☐☐☐

TITLE

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: Evaluation of BiologicalAssimilatory Capacity for Mechanism-Based Adaptive Management for Selenium in the San **Franciso** Bay-Delta

12. AREAS AFFECTED BY **PROJECT** *(Cities, Counties. States, etc.):* California

13. PROPOSED PROJECT

14. CONGRESSIONAL DISTRICTS **0 F**

Start Date	Ending **Date**	a. Applicant	b. Project
Sep 1,2000	**Aug** 31.2003	Third	Third

15. ESTIMATEDNNDING — First year

a. Federal	$ 214,009 00
b. Applicant	$ m
c. Slate	$ m
d. Local	$ 00
e. Other	$ 00
f. Program Income	$ m
g. TOTAL	$ 214.009 00

16. **IS** APPLICATION **SUBJECT TO REVIEW** BY **STATE** EXECUTIVE ORDER 12372 PROCESS?

a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE _____

b. NO. ☒ PROGRAM IS NOT COVERED BY E. 0.12372
☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?

☐ Yes If "Yes," attach an explanation. ☒ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA **IN** THIS **APPLICATION/PREAPPLICATION** ARE TRUE AND CORRECT, THE DOCUMENT **HAS** BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE **APPLICANT** WILL COMPLY **WITH THE ATTACHED** ASSURANCES IF THE ASSISTANCE **IS** AWARDED.

a. Type Name of Authorized Representative	b. Title Sandra M. Dowdy Contracts and Grants Analyst	c. Telephone Number (530) 752-2075
d. Signature of Authotized Representative Sandra M. Dowdy		e. Date signed MAY 1 1 2000

Previous Edition Usable
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Standard Form 424 (Rev. 7-97)
Prescribed by OMB Circular A-102

OMB Approval No. 0348-0044

BUDGET INFORMATION - Non-Construction Programs

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1 Se Forms		$	$	$ 326,522	$	$ 326,522
2. Fish Histopathology				324,766		324,766
3.						
4.						
5. Totals		$	$	$ 651,288	$	$ 651,288

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1) Se Forms	(2) Fish Histopathology	(3)	(4)	Total (5)
a. Personnel	$ 209,804	189,753	$	$	$ 399,557
b. Fringe Benefits	42,033	38,015			80,048
c. Travel	6,000	3,000			9,000
d. Equipment	0	8,500			8,500
e. Supplies	39,000	56,745			95,745
f. Contractual	0	0			0
g. Construction	0	0			0
h. Other	0	0			0
i. Total Direct Charges (sum of 6a-6h)	296,837	296,013			592,850
j. Indirect Charges 10% of direct costs less equipment	29,685	28.753			58,438
k. **TOTALS (sum** of 6i and 6j)	$ 326,522	$ 324,766	$	$	$ 651,288
7. Program income	$	$	$	$	$

SECTION C - NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8.	$	$	$	$
9.				
10.				
11.				
12. TOTAL (sum of lines 8 - 11)	$ 0	$ 0	$ 0	$ 0

SECTION D - FORCASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$ 214,009	$ 59,878	$ 51,377	$ 51,377	$ 51,377
14. NonFederal					
15. TOTAL (sum of lines 13 and 14)	214,009	59,878	51,377	51,377	51,377

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (Years) (b) First	(c) Second	(d) Third	(e) Fourth
16. Se Fomrs	$ 107,642	$ 113,062	$	$
17. Fish Histopathology	105,317	111,258		
18.				
19.				
20. TOTAL (sum of lines 16-19)	$ 212,959	$ 224,320	$	$

SECTION F - OTHER BUDGET INFORMATION

21. Direct Charges: 592,850

22. Indirect Charges: 10% of direct costs less equipment 58,438

23. Remarks: Indirect costs, 10%, are based on funds from California State Resources Agency. Other agencies may be higher overhead. The overhead rates for Federal agencies are 46.5, 48.0, and 48.5% for years 1, 2, and 3, respectively, yielding a total budget of $871,559.

STATE OF CALIFORNIA

NONDISCRIMINATION COMPLIANCE STATEMENT

STD. 19 (REV. 3-95)

COMPANY NAME

The company named above (herinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, physical disability (including HIV and AIDS), medical condition (cancer), age (over 40), marital status, denial of family care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I amfully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

OFFICIAL'S NAME: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

DATE EXECUTED: MAY 1 1 2000

EXECUTED IN THE COUNTY OF: Yolo

PROSPECTIVE CONTRACTOR'S SIGNATURE: Sandra M. Dowdy

PROSPECTIVE CONTRACTOR'S TITLE: Sandra M. Dowdy
Contracts and Grants Analyst

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME

OMB Approval No. 0348-0040

ASSURANCES - NON-CONSTRUCTION PROGRAMS

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

AS the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related *to* the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973. as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps: (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§6101-6107), which prohibits discrimination on the basis of age: (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse: (9 the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism: (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee 3), as amended, relating *to* confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing: (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. .Will comply, as applicable, with provisions of the Hatch Act *(5* U.S.C. $51501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will 'comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 16 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of' the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11968; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and. (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1966 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and "the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).

14. Will comply with P.L 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits *in* accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, 'Audits of States, Local Governments, and Non-Profit Organizations.'

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
Sandra M. Dowdy	Sandra M. Dowdy Contracts and Grants Analyst
APPLICANT ORGANIZATION	DATE SUBMITTED
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	MAY 11 2000

U.S. Department of the Interior

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used; use this form for certification and sign; or use Department of the Interior Form 1954 (DI-1954). (*See* Appendix A of Subpart D of *43* CFR Part 12.)

Certification Regarding Debarment, Suspension. Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of *43* CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (*See* Appendix C of Subpart D of 43 CFR Part 12.)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A: Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions

---

CHECK — IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment. declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency:

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery. bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

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CHECK __ IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

01-2010
March 1995
(This form consolidates DI-1953. DI-1954, DI-1955. DI-1956 and DI-1963)

PART C Certification Regarding **Drug-Free** Workplace Requirements

CHECK __ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL

Alternate **I.** (Grantees Other Than Individuals)

A The grantee certifies that it will or continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution. dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace:
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a):

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will –
(1) Abide by the terms of the statement; and
(2) Notify the employer in writing of tis or her conviction for a violation of a criminal drug statute occurring in the'workplace no later than five calendar days after such conv lction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction, Employers of convicted employees. must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted –
(1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of **1973,** as amended; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal; State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b). (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)

Univ. of California
Hazing and Hospitality Hall
Yolo County, Davis, CA 95616

Check __ if there are workplaces on file that are not identified here.

PART D: Certification Regarding **Drug-Free** Workplace Requirements

CHECK __ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

(a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution. dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

PART E: Certification Regarding Lobbying
Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK __ IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS $100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT, SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK __ IF CERTIFICATION IS FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF $150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING $100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undesigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan. or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connectionwith this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction vias made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31. U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL Sandra M. Dowdy

TYPED NAME AND TITLE Sandra M. Dowdy
Contracts and Grants Analyst

DATE MAY 11 2000

DI-2010

March 1995

(This form consolidates DI-1953, DI-1954, DI-1955. DI-1956 and DI-1963)

Environmental Compliance Checklist

All applicants must fill out **this** Environmental Compliance Checklist. Applications must contain answers to the following questions to be responsive and to be considered **for** funding. ***Failure to answer these questions and inclu.dethem with the application will result in the application being considered nonresponsive and not considered for funding.***

1. **Do** any **of** the actions included in the proposal require compliance with either the California Environmental Quality Act (CEQA), the National Environmental Policy Act (NEPA), or both?

   ____ YES X NO

2. If ynu answered yes to # 1, identify the lead governmental agency for CEQA/NEPA compliance.

   _____
   Lead Agency

3. If you answered no **to** # **1,** explain why CEQA/NEPA compliance is no! required **for** the actions in the **proposal.**

   Research proposed is mechanistic in nature, involving laboratory-raised fish and invertebrates, histopathological and biochemical analyses. Purpose is to establish biomarkers of exceedance of biological assimilatory capacity for Se.

4. **If** CEQA/NEPA compliance is required, describe how the project will comply with either or both of these laws. Describe where the project **is** in **the** compliance process and the expected date **of** completion.

5. Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?

   ____ **YES** X NO

   If yes, the applicant must attach written permission for access from the relevant property owner(s). Failure **to** include written permission **lor** access may result **in** disqualification of the proposal during the review process. Research and monitoring field projects for which specific field locations have not been identified **will** be required to provide access needs and permission for access with **30** days **of** notification of approval.

6. Please indicate what permits **or** other approvals may **be** required **for** the activities contained **in** your proposal. Check all **boxes** that apply.

LOCAL		
Conditional use permit	___	
Variance	___	
Subdivision Map Act approval	___	
Grading permit	___	
General plan amendment	___	
Specific plan approval	___	
Rezone	___	
Williamson Act Contract cancellation	___	
Other _____ (please specify)		
None required	X	
STATE		
CESA Compliance	___	(CDFG)
Streambed alteration permit	___	(CDFG)
CWA § 401 certification	___	(RWQCB)
Coastal development permit	___	(Coastal Commission/BCDC)
Reclamation Board approval	___	
Notification	___	(DPC, BCDC)
Other _____ (please specify)		
None required	X	
FEDERAL		
ESA Consultation	___	**(USFWS)**
Rivers & Harbors Act permit	___	(ACOE)
CWA § **404** permit	___	(ACOE)
Other _____ (please specify)		
None required	X	

DPC = Delta Protection Commission
CWA = Clean Water Act
CESA = California Endangered Species Act
LISFWS = U.S. **Fish** and Wildlife Service
ACOE = U.S. Army Corps of Engineers

ESA = Endangered Species Act
CDFG = California Department of Fish and Game
RWQCB = Regional Water Quality Control Board
BCDC = Bay Conservation and Development Comm.

Land Use Checklist

All applicants must fill out this Land Use Checklist for their proposal. Applications must contain **answers** to the following questions to be responsive **and** to be considered for funding. ***Failure to answer these questions and include them with the application will result in the application being considered nonresaonsive and not considered for funding.***

1. Do the actions in the proposal involve physical changes to the land (i.e. grading, planting vegetation, or breeching levees) or restrictions in land use (i.e. conservation easement or placement **of** land in a wildlife refuge)?

_____ **YES** X **NO**

2. If NO to # **1,** explain what type **of** actions are involved in the proposal (i.e., research only, planning only).

The project is research only.

3. If YES to # 1, what **is** the proposed land use change **or** restriction under the proposal?

4. If YES to # **1,** is the land currently under a Williamson Act contract?

_____ YES _____ **NO**

5. If YES to # **1,** answer the following:

Current land use _____
Current zoning _____
Current general plan designation _____

6. **If** YES to #1, is the land classified as Prime Farmland, Farmland **of** Statewide Importance or Unique Farmland **on** the Department **of** Conservation Important Farmland Maps?

_____ YES _____ **NO** _____ **DON'T KNOW**

7. **If** YES to # I, how **many** acres **of land will** he subject to physical change or land use restrictions under the proposal?
_____

8. **If YES to** # **1,** is the property currently being commercially farmed ***or*** grazed?

_____ YES _____ **NO**

9. **If** YES to #8, what are the number **of** employees/acre _____
the total number **of** employees _____

10. **Will the** applicant acquire any interest in land under the proposal **(fee** title **or** a conservation easement)?

_____	**X**
YES	**NO**

11. **What** entity/organization will hold the interest?_____

12. If **YES** to **# 10,** answer the following:

Total number **of** acres **to** be acquired under proposal _____
Number of acres **to be** acquired in fee _____
Number of acres to **be** subject to conservation easement _____

13. For **all** proposals involving physical changes to the laud **or** restriction in land **use,** describe what entity or organization will:

manage the property _____

provide operations and maintenance services _____

conduct monitoring _____

14. **For** land acquisitions (fee title **or** easements), will existing water rights also **be** acquired?

_____	_____
YES	**NO**

15. **Does the** applicant propose any modifications **to** the water right or change in the delivery **of** the water?

_____	**X**
YES	**NO**

16. **If YES** to **# 15,** describe_____